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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:
DE BOER, Mark, et al.

Serial No.: 09/235,073

Filed: January 21, 1999

For: INDUCTION OF T-CELL TOLERANCE WITH CD40/B7 ANTAGONISTS

Group Art Unit: 1645

Examiner: R. Zeman

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

DECLARATION UNDER 37 C.F.R. § 1.132 OF
Dr. Louis Boon

I, Louis Boon, Ph.D., declare:

1. I am a citizen of The Netherlands and reside at Sandinostraat 9 1069 NJ Amsterdam.

2. I received a doctorate degree in Chemistry in 1991.

3. I have been employed at Tanox Pharma B.V. (a subsidiary of Tanox, Inc.) since 1995 as a scientist.

4. I designed experiments to investigate whether there was an additional immunosuppressive effect, indicating an additional potential therapeutic benefit when chimeric 5D12 (anti-CD40) and chimeric FUN-1 (anti-CD86) were administered in combination over the results obtained when ch5D12 was administered alone.

5. The combination of antagonist MAbs (anti-human CD40 and anti-human CD86) or anti-CD40 antagonist MAb alone was administered to rhesus monkeys to effect transient immunosuppression. The animals were also intravenously injected with a recombinant E1/E3-deleted adenovirus vector. These adenovirus vectors contained as transgenes either human soluble CD4-Ig (hu-sCD4) driven by an RSV promoter or murine IFN-γ (mu-IFN-γ) driven by a CMV promoter. Control animals received an adenovirus vector, but did not receive any antibody. The four experimental animals and the four control animals all received an adenovirus injection at day 0 and at day 32. Experimental animals also received 10 mg/kg MAb at day -3, and 5mg/kg MAb administered at day 0 and day 3. Two experimental animals received anti-CD40 alone, and the other two experimental animals received the combination treatment of anti-CD40 and anti-CD86.

6. Figures 1 and 2 illustrate the effects, following adenovirus vector administration, on the expression of hu-sCD4, mu-IFN-γ, and the production of neutralizing anti-adenovirus antibodies in the control animals. The expression of the transgene in the first injected adenovirus vector (respectively, mu-IFN-γ (Figure 1) or hu-sCD4 (Figure 2)), was transient, and serum levels of such transgene were reduced to an undetectable level by day 10-35. Significant production of anti-adeno neutralizing antibodies followed this first adenovirus vector administration, and the expression of the transgene present on the second injected adenovirus vector (respectively, hu-sCD4 (Figure 1) or mu-IFN-γ (Figure 2)) was completely abolished. All of the control animals exhibited a strong humoral anti-adenovirus response following the first vector

administration as evidenced by the level of neutralizing antibody titers. Figures 1 and 2 show that the rise in neutralizing anti-adeno antibodies was most dramatic between days 3 and 7 post-injection for 3 of the 4 control animals. High total antibody levels (data not shown), as well as high neutralizing antibody titers, were observed in all four animals prior to administration of the second adenovirus vector. As expected, due to the presence of high titers of the anti-adeno neutralizing antibodies, administration of the second adenovirus vector was ineffective and no second transgene product was detected in the serum samples (Figures 1 and 2).

7. In the experimental group, two animals were treated with chimeric anti-CD40 MAb (ch5D12) alone, and two other animals were treated with a combination of ch5D12 and chimeric anti-CD86 MAb (chFun-1) at days –3 (10 mg/kg, for each Mab), 0 (5 mg/kg, for each Mab), and +3 (5 mg/kg, for each Mab) relative to the first adenovirus administration. Animals 1, 2, and 4 received the same dose of adenovirus, whereas animal 3, treated with the combination of ch5D12 and chFun-1, received a 3-fold lower dose of adenovirus in the first administration. Expression of the first transgene (hu-sCD4) was significantly prolonged in animal 3 (Figure 4), presumably due to the lower level of neutralizing antibodies produced in response to this 3-fold lower dose of the adenovirus (See ¶ 9). As expected, this animal showed a lower initial transgene expression level than the 3 other animals, but, surprisingly, a higher level of expression at day 50 than animal 4, which also received the combination of antibodies, and higher than animals receiving anti-CD40 MAb alone. The expression level of the first transgene (hu-sCD4) in animal 4 indicates that very high doses of adenovirus

vector may activate the animal's immune response and neutralize transgene expression product more effectively than lower doses (animal 3).

8. All four experimental animals treated with MAb showed detectable levels of the first transgene product, hu-sCD4, in the serum (Figures 3 and 4). In contrast to the control animals however, mu-IFN-γ expression was detected in all four experimental monkeys following administration of the second adenovirus vector at day 32 (Figures 3 and 4). Surprisingly, in the animals treated with ch5D12 alone, mu-IFN-γ was detectable only in trace amounts (less than 5 pg/ml) as compared to 300 pg/ml in animal 4 and > 3000 pg/ml in animal 3 treated with the combination. Also, animal 3 had a 5000-fold higher expression of mu-IFN-γ than the monkeys treated with ch5D12 alone. Animal 3 had no detectable level of neutralizing antibodies before the second adenovirus injection (Figure 5).

9. Analysis of the humoral response against adenovirus following the first administration is presented in Figure 5. Results show a prolonged suppression of the level of neutralizing antibodies against the adenovirus particles in the experimental monkeys receiving ch5D12 and the combination ch5D12 + chFun-1 as compared to the control monkeys.

10. The significantly higher level of expression of the second transgene in the two monkeys receiving the combination of an anti-CD40 MAb and an anti-CD86 MAb demonstrates the effectiveness of this combination over anti-CD40 alone. The added effectiveness of the combination is probably due to blocking both the CD28 and the CD40 pathways versus blocking only the CD40 pathway anti-CD40 alone.

11. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: August 13th

Dr. Louis Boon